NCER-EPA STAR Annual Report Summary

Date of Report: 5/1/2009

EPA Agreement Number: EPA-G2006-STAR-M1

Title: Fate of hormones in tile-drained fields and impact to aquatic organisms under different animal waste land-application practices

Investigators: Linda S. Lee (lslee@purdue.edu; http://www.agry.purdue.edu/staffbio/lslbio.htm)
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Research Category: Fate and Effects of Hormones in Waste from CAFOs

Project Period: April 16, 2007 – April 15, 2010

Objective of Research: (1) Assess the relative amount of hormones discharged from tile-drained agricultural fields under different manure and lagoon effluent applications; (2) Assess hormone persistence in fields under these application practices; and (3) Evaluate the impacts of these hormone loads (relevant levels and mixtures) on aquatic organisms.

Progress Summary/Accomplishments:

Animal Science Research and Education Facility (ASREC) and Water Quality Field Station (WQFS) Sites: We set up a wireless communication system of all stations at the ASREC site (and one representative station at the WQFS site) and completed development of a recession model, which is allowing us to reduce the number of samples needed to adequately describe the rise and fall of each hydrograph. Water samples from both drains and stream stations are being collected and analyzed for 17α- and 17 β-estradiol and trenbolone, and related metabolites (estrone, estriol, trendione, androstendione) using LC/MS. A subset of samples from both drains and streams are being analyzed for pH, EC, nitrates, phosphate, and dissolved organic carbon; and a subset of stream samples are being analyzed for pesticides used on the associated fields. Chemical and flow data are being complied and evaluated in light of management activities, landscape details, and weather conditions. From September 2008 through February 2009 our LC/MS/MS detector was in operable due to some unusual circumstances that were started by a planned power outage. Purdue insurance agreed to replace the MS, which occurred in February and became fully operational in March.

Laboratory-based Degradation Studies: Further studies were conducted on the persistence of the major metabolites of 17β-trenbolone acetate (TBA) present in beef manure being land-applied (17α-trenbolone and trendione) under a range of temperature and moisture conditions of environmental relevance. Under temperatures favorable for microbial activity, degradation rates decreased with decreasing water availability, e.g., at 25 °C, rates decreased from 0.18 h^{-1} (-0.3 bar) to 0.08 h^{-1} (-5 bar). Under favorable water availability, degradation rates decreased with decreasing temperature. Microbial activity at 5C was always very low.

Persistence in Beef-Manure and Effluent: We quantified the concentrations of TBA, testosterone, and estradiol metabolites in beef manure in manure and manure-receiving lagoon water for 9 weeks after cattle were administered TBA/estradiol implants. Beef cattle here are implanted with commercially available anabolic preparation *Ravoler-S* (containing 140 mg of trenbolone acetate and 28 mg of estradiol). In manure slurry, concentration patterns generally followed a rise and fall pattern, except for 17β-estradiol, with the timing of peak concentration hormone specific. 17α-trenbolone was the most abundant androgen at all times with the highest concentration observed the 4th week after implant (3.6 mg/kg manure). Trendione and 17β-trenbolone peaked at ~0.4 and ~ 0.2 mg/kg at the end of week 4 and 2, respectively. For the estrogens, the highest concentrations were observed for the metabolites estrone (3 mg/kg after week 4) and estriol (2.2 mg/kg after week 6). The highest concentrations observed for 17α-estradiol in manure was after week 4 at 0.6 mg/kg.17β-estradiol concentrations appeared erratic over time between ~ 0.1 to 0.1 mg/kg, which may be due to some reversible conversion from estrone that can occur

under anaerobic conditions. In lagoon water, 17α-trenbolone and estrone were detected in highest concentrations (1500 and 1700 ng/L respectively).

Hormone Impact on Aquatic Organisms: Turtles were collected for an additional reproductive season (May-June) in 2008 and results were similar to those observe din the 2007c collection. In relation to fish studies, sampling was conducted along two agricultural ditches (Marshall and Box Ditches) and one control site (Ghost Creek). Marshall Ditch is bordered by crop production (corn and soybean) and receives field runoff treated with manure from beef and dairy concentrated animal feeding operations (CAFO). Box Ditch is solely crop production, but has drainage from Marshall Ditch. Ghost Creek runs through a recreational area and is bordered by a small shrub and tree cover. Each site was sampled, using electro fishing via DC pulse on three distinct transects, every other month in 2008 starting in May throughout September. Fish collected were identified to species and measured prior to release. Reproductive condition of creek chubs, *Semotilus atromaculatus*, a species commonly found in all three sites, was also assessed. Results revealed lower species richness and fish numbers in both agricultural ditches compared to that of the control. These results imply that current ditch management practices have negative impacts on aquatic fauna.

Publications*(*denotes graduate or undergraduate student)***:**

Khan*, B., S.A. Sassman, and **L.S. Lee**. 2008. Degradation 17α- and 17β-Trenbolone and Trendione in Agricultural Soils. *Environ. Sci. Technol.*, 42:3570-3574.

*Gall, Heather. 2008. Real-Time Monitoring and Automated Sampling of Purdue's Agricultural Fields. MS Thesis, Purdue University (December 2008).

Abstracts:

*McAlexander A, Lee L, Goforth R, **Sepúlveda M**. 2008. Impacts of concentrated animal feeding operations on fish communities. 69th Midwest Fish and Wildlife Conference, December 14-17, Columbus, OH (poster).

Laessig SA, Ankley G, Durhan E, Gray LE, Hutchins S, Lazorchak J, Martinovic D, Mills M, Wilson V, Landy R, Fisher J, Kolodziej E, **Lee L**, Sedlak D, Snow D, **Sepúlveda M**, Yonkos L. 2008. Collaborative studies on occurrence and risk of estrogens and androgens in discharges from concentrated animal feeding operations. USEPA STAR Endocrine Disruptor Program. Tampa, FL (oral).

Durhan E, Wilson V, **Sepúlveda M**, Martinović D, **Lee L**, Lazorchak J, Lambright C, Cavallin J, Blake L, Ankley G. 2008. Newer analytical and fractionation approaches for detecting endocrine-active chemicals in CAFOs. SETAC 29th Annual Meeting, November 16-20, Tampa, FL (poster).

*McAlexander A, Lee L, Goforth R, **Sepúlveda M**. 2009. Impacts of concentrated animal feeding operations on fish communities. Joint Meeting of the Indiana Chapter of the American Fisheries Soc. and the Indiana Lake Management Soc., Jan 29-31, Indianapolis, IN (***Awarded best student poster.***

*Leet J, Amberg J, **Sepúlveda M**. 2009. Sex determination in fathead minnows (*Pimephales promelas*) larvae. FNR Annual Research Symposium, April 17, West Lafayette, IN (poster).

*Rogers S, Meyer J, **Lee L**, **Sepúlveda M**. 2009. Plasma vitellogenin levels in common snapping turtles (*Chelydra serpentina*) from concentrated animal feeding operation (CAFO) ponds versus a reference site. 2009. FNRs Annual Research Symposium, April 17, West Lafayette, IN (poster). ***Awarded third price for undergraduate student poster.***

*Rogers S, Meyer J, **Lee L**, **Sepúlveda M**. 2009. Plasma vitellogenin levels in common snapping turtles (*Chelydra serpentina*) from concentrated animal feeding operation (CAFO) ponds versus a reference site. 2009. College of Undergraduate Research Symposium, April 21, West Lafayette, IN (poster).

Gall*, H.E., **Jafvert, C.T., Lee, L.S**., Jenkinson, B. "Real-Time Monitoring and Automated Sampling of Tile-Drains and Near-Field Streams," poster presented at the Ohio Valley Chapter of Science of Environmental Toxicology and Chemistry (SETAC), Oct. 3, 2008.

Khan*, B. and **L.S. Lee**. 2008. Soil Temperature and Moisture Effects on Persistence of 17α-trenbolone and Trendione. SETAC North America 29th Annual Meeting, November 16-20, Tampa, FL.

Lee, L. S. and B. Khan*. 2008. Stereo selective sorption of trenbolone and estradiol in agricultural soils (No. 61-5). 2008 Joint Meeting of The Geological Society of America, Soil Science Society of

America, American Society of Agronomy, Crop Science Society of America, Gulf Coast Association of Geological Societies with the Gulf Coast Section of SEPM, Houston, TX, USA.

Future Activities: Collection and analysis of water samples from both ASREC and WQFS drains and ASREC stream stations will continue as well as compilation of chemical and flow data in an ACCESS database along with management activities, landscape details, and weather conditions. We will complete our field and lab studies on the impact to fish and turtles of agricultural drainage form manure-applied fields.

Supplemental Keywords: endocrine disrupting chemicals, ecological effects, aquatic indicators, fish gonadal changes, sex differentiation, ecosystem health risks, manure, effluent irrigation, subsurface injection, broadcasting, androgens, estrogens, trenbolone, trendione, testosterone, estradiol, estrone, estriol, degradation, discharge, chemical transport, soil

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Institution: Purdue University, West Lafayette, IN 47907

Research Category: Fate and Effects of Hormones in Waste from CAFOs

Project Period: April 16, 2007 – April 15, 2010

Objective of Research: (1) Assess the relative amount of hormones discharged from tile-drained agricultural fields under different manure and lagoon effluent applications; (2) Assess hormone persistence in fields under these application practices; and (3) Evaluate the impacts of these hormone loads (relevant levels and mixtures) on aquatic organisms.

Progress Summary/Accomplishments:

Animal Science Research and Education Facility (ASREC) Site: During the first year of the project we established four tile drain and three stream/ditch sampling and flow monitoring stations at the ASREC site. We began water sample collection as each station that was in full operation. Samples are being processed and analyzed for 17α- and 17β- estradiol and trenbolone, and related metabolites (estrone, estriol, trendione, androstendione) using LC/MS. A subset of samples from both drains and streams are also being analyzed for pH, EC, nitrates, phosphate, and dissolved organic carbon; and a subset of stream samples are being analyzed for pesticides used on the associated fields.

Water Quality Field Station (WQFS) Site: We initiated flow-weighted sampling of drainage from eight 520 m^2 plots fertilized with subsurface swine manure injection (4 in the spring prior to planting and 4 in the fall after harvest) at our already established WQFS. 2007 data from this site showed the presence of primarily estrogens in discharge water in response to rain events that occurred approximately three weeks after subsurface injection of swine manure, but all concentrations were lower than concentrations known to invoke negative responses to aquatic species

Hormone Persistence in Manure-Applied Fields: We attempted to quantify persistence of hormones in manure-applied fields by subsampling fields subsurface injected with swine manure; however, we encountered several problems. There were extreme variability between subsamples and challenges with limits of quantitation (LOQ) in the samples during the first 2 weeks after application. LOQ were 3 orders of magnitude higher for water collected from the tile drains such that we may see no quantifiable limits in the field soil-manure subsamples, but continue to see substantial hormone concentrations in the aqueous discharge. Therefore, we chose to focus on assessing persistence in laboratory microcosm studies under a range of temperature and moisture conditions that would represent field conditions. For the hormones of interest, no literature was available on persistence of metabolites excreted from 17β-trenbolone acetate (TBA)-implanted cattle. TBA is converted metabolically to primarily 17α-trenbolone and trendione, and excreted in manure from implanted cattle. To predict the persistence of TBA metabolites once land-applied via manure, aerobic degradation rates in two contrasting agricultural soil types (clay loam and a sandy soil) of both trenbolone isomers (17α and 17β) and their primary metabolite trendione were measured and isomer interconversion was assessed. The impact of manure application was also evaluated in the clay loam soil. At 25°C and optimal moisture conditions, both isomers degraded to trendione in a similar manner with half lives ($t_{½}$) in the order of a few hours to 0.5 d at applied concentrations of ≤ 1 mg/kg. Similar degradation rates were also observed in the presence and absence of manure applied at

rates typical for land-application of cattle manure. Trenbolone degradation was concentration-dependent with degradation rates decreasing with increasing applied concentrations. Trendione, whether applied directly or produced from trenbolone, persisted longer than trenbolone with $t_{½}$ values of 1 to 4 d. A small amount (1.5%) of conversion of trendione back to 17β-trenbolone was observed during aerobic incubation regardless of the applied concentration. A small amount of 17α–isomer also converted back to 17β-trenbolone, presumably through trendione. In autoclaved soils, degradation was small to negligible compared to a microbially active soil.

Sorption Studies: To aid in predicting transport of manure-borne TBA metabolites, multi-concentration sorption isotherms for 17α- and 17β-trenbolone and trendione were generated with five autoclaved-sterilized soils that represented a range in soil properties. Hormone concentrations were measured independently in solution and soil phases, and quantified using LC/MS.

Hormone Impact on Aquatic Organisms:
We have sampled fish and turtles from streams and ponds receiving runoff from fields treated with animal manure. In order to determine exposure to estrogens in turtles, we have developed methods for the semi-quantification of VTG in plasma of common snapping turtles (Chelydra serpentina). Turtles have been collected during the reproductive season (May-June) in 2007. VTG was detected in plasma samples from all females but absent from male turtles from both reference and CAFO sites. Comparison of the relative abundance of VTG plasma concentration showed that the average VTG concentrations were similar between the two female populations, but that relative VTG concentrations were more variable in the population from the CAFO site than the population from the reference site. In spring 2008 we began exposing fathead minnows (from day 0 to day 30 post-fertilization) to water from Marshall Ditch in a flow-through tank system built inside a small shed. We had mixed results in that our survival was low.

Publications/Presentations *(*denotes graduate student)*:

Lee, L.S. Livestock and Poultry Education (LPE) Pharmaceutical Team – contributed to pieces for web posting on a National website in Antibiotic Fate, Transport, Sources, and Impacts. Webinar on Fate & Transport of Hormones & Antimicrobials, April 25, 2008 (attendance ~ 90)

Lee, L.S. Fate & Transport of Antibiotics, February 4, 2008, CREES National Water Conference

Khan*, B., Sassman, S.A. and **Lee, L.S**. 2007. Degradation of 17α- and 17β-Trenbolone and Trendione in Agricultural Soils, Ecological Science & Engineering Graduate Student Symposium, Purdue University, March 2007.

Future Activities:
We will be setting up a wireless communication system at all of the ASREC stations and initiate development of a recession model that would allow us to optimize sample collection during the rise and fall of each hydrograph, which would reduce our overall analysis and labor costs without compromising chemograph construction. Collection and analysis of water samples from both ASREC and WQFS drains and ASREC stream stations will continue. In addition, a subset of samples from both drains and streams are being analyzed for pH, EC, nitrates, phosphate, and dissolved organic carbon; and a subset of stream samples are being analyzed for pesticides used on the associated fields. We will also begin to compile chemical and flow data in an ACCESS database along with management activities, landscape details, and weather conditions. We will extend our TBA metabolite persistence study to include a range of temperature and moisture conditions of environmental relevance. We will continue to sample fish and turtles from streams and ponds receiving runoff from fields treated with animal manure. In collaboration with USEPA Duluth, hormone concentrations will also be measured using radioimmunoassay in each of the plasma samples.

Supplemental Keywords: endocrine disrupting chemicals, ecological effects, aquatic indicators, fish gonadal changes, sex differentiation, ecosystem health risks, manure, effluent irrigation, subsurface injection, broadcasting, androgens, estrogens, trenbolone, trendione, testosterone, estradiol, estrone, estriol, degradation, discharge, chemical transport, soil

Executive Summary

Date of Final Report: **11/11/2011**

EPA Agreement Number: EPA-G2006-STAR-M1, Grant # RD833417

Title: Fate of hormones in tile-drained fields and impact to aquatic organisms under different animal waste land-application practices

Investigators: Linda S. Lee (lslee@purdue.edu); Marisol S. Sepúlveda (mssepulv@purdue.edu); Chad T. Jafvert (jafvert@ecn.purdue.edu)

Institution: Purdue University, West Lafayette, IN 47907

Project Period: April 16, 2007 – April 15, 2011

Project Amount: $750,000

RFA: RFA-DK-06-004

Research Category: Fate and Effects of Hormones in Waste from CAFOs

Description and Objective of Research: The overall goal of this project was to evaluate hormone discharges from subsurface tile drains of agricultural fields treated with liquid and solid animal wastes and assess their impacts on aquatic species in the drainage receiving ditch network. Specific objectives included (1) assessing the relative amount of hormones discharged from tile-drained agricultural fields under different manure and lagoon effluent applications; (2) assessing hormone persistence in fields under these application practices; and (3) evaluating the impacts of these hormone loads (relevant levels and mixtures) on aquatic organisms.

Field monitoring studies were conducted to evaluate hormones in subsurface tile drainage and the ditch network receiving drainage from fields at an EPA-designated CAFO in Northern Indiana, which has approximately 600 ha of tile drained cropland treated with various types of animal wastes (beef, dairy, and poultry lagoon effluent, dairy solids, and subsurface injection of swine manure). Seven sampling stations (four in tile drains and three in the receiving ditch network) were installed and used to monitor flow continuously and collect water samples during storm events and baseline flow for a 17-month study period. Natural hormones 17α- and 17β-estradiol (E2), estrone (E1), estriol (E3), testosterone, and androstenedione and the synthetic androgens 17α- and 17β-trenbolone and trendione were monitored. Additional parameters such as pH, EC, nitrate, ammonia, ortho-P, selected pesticides, and dissolved organic carbon (DOC) were also measured on a subset of stream and tile drainage samples. The effects of CAFO impacted streams and ponds sites compared to reference sites were evaluated for abundance and diversity of fish (fish community study) and growth and reproductive conditions. Sexual differentiation and reproductive effects were also evaluated by exposing early life-stages of fathead minnows *in situ* to CAFO ditch water hormones. These studies were paired with field studies examining similar endpoints in native populations of fish in streams down gradient from CAFOs and in turtles inhabiting manure retention lagoons.

SUMMARY OF FINDINGS

Our analysis of the monitoring work conducted from January 2009 to May 2010 focused on assessing hormone concentrations, fluxes, and exports during storm events, base flow, thawing/snow melt events, and effluent irrigation in subsurface tile drains and the ditch network receiving drainage from fields treated with various types of animal wastes. Hormones were detected in ~60% of the samples collected at each station during the 17-mo study, with estrone being detected the most frequently and estriol the least. Testosterone and androstenedione were detected more frequently than synthetic androgens, which were detected in fewer than 15% of the samples. Hormone concentrations in subsurface tile drains increased during effluent irrigation and storm events. Hormones also appeared to persist over the winter, with increased concentrations coinciding with early thaws and snow melt from fields applied with manure solids. The highest concentration of synthetic androgens (168 ng/L) observed coincided with a snow melt. The highest concentrations of hormones in the ditch waters (87 ng/L for total estrogens and 52 ng/L for natural androgens) were observed in June, which coincides with the early life stage development period of many aquatic species in the Midwest.

Hormone fluxes were highest during storm events shortly following animal waste applications with flow-weighted concentrations generally decreasing during subsequent storm events prior to additional applications. The total estrogen fluxes from fields were highest during large spring storm events between May and June 2009 following dairy effluent irrigation and dairy solids applications. Hormone loads exported from the receiving ditch network ranged from 16 – 58 mg/ha for total estrogens, 6.8 – 19 mg/ha for natural androgens, and 4.2 - 44 mg/ha for synthetic androgens. High temporal variability in hormone export led to the majority of hormone loads occurring when flow rates were in the 80^{th} percentile. High export during the spring months has the potential to negatively impact sensitive aquatic ecosystems downstream, as it coincides with the sensitive early life stages of many aquatic species. Therefore, our findings suggest that the short periods of time during which high-flow events occur must be targeted to effectively reduce hormone loads exported to downstream aquatic ecosystems.

From our sorption and degradation studies, isomeric-specific degradation was not evident. However, sorption is isomeric-specific with the α isomer sorbing less by approximately a factor of two. Therefore, α and β isomers sorb the same is not a conservative decision making approach. The lower sorption affinity of the α-isomer increases its likelihood to be transported easily to streams and rivers from agricultural fields, which is significant given it is the dominant TBA metabolite in manure and has been observed to have similar aquatic reproductive effects as 17β-trenbolone and likewise for 17α-E2 for some animal species.

Fish species diversity, intolerant species, and the index of biological integrity (IBI) showed significantly lower species richness and IBI for the two CAFO impacted agricultural ditches compared to the reference site. Dissolved oxygen was more consistent at the reference site compared to the CAFO impacted sites, but the means were not statistically different; however, water temperatures were 4 to 5 °C higher at the reference site. During field sampling for the fish community study in late April/early May and mid-June, 12 creek chub were randomly collected from each of the three sites to assess a gonadosomatic index (GSI), reproductive conditions, and reproductive stage. Somatic growth appeared faster at one of the CAFO impacted sites compared to the reference site as well as significant differences in reproductive conditions. Throughout the

summer months, water levels declined and temperatures increased in the agricultural ditches with production of algal blooms, which severely reduced levels of dissolved oxygen throughout the summer. This resulted in an even a higher decline in fish species richness and abundance. In comparison, the control site retained higher dissolved oxygen levels, higher total numbers of fish, and greater species richness. These results imply that current ditch management practices have negative impacts on aquatic fauna.

Fathead minnow embryos exposed to CAFO-impacted ditch water in spring-summer 2009 showed a higher % of males (~58 %) relative to the control populations, but not of statistical significance. In collaboration with USEPA Cincinnati and Duluth laboratories, we conducted. *In-situ* caging studies deploying adult fathead minnows in our CAFO and reference sites for a week in early June showed no significant differences in their relative expression of vitellogenin (VTG, egg yolk protein). Differences in plasma VTG concentrations in plasma samples collected from common snapping turtles (32 were recorded as male and 19 were recorded as female) over the three summers from 2007-2009, were also not statistically significant between CAFO-impacted and reference sites. However, male turtles at the CAFO impacted poultry pond did have significantly higher testosterone than those at reference pond.

CONCLUSIONS

Hormones appeared to persist over the winter, with increased concentrations coinciding with early thaws and snow melt from fields applied with manure solids. However, hormone fluxes and loads were highest during large spring storm events between May and June 2009 following animal waste applications. The majority of hormone export occurred when flow rates were in the 80^{th} percentile of the flows experienced throughout the year. Due to this significant temporal inequality exhibited by hormone export, best management practices must be aimed at reducing export during high-flow events to achieve significant reductions in hormone loads. If these "windows of opportunity" are missed, then any efforts to mitigate loads during all other periods of time will have minimal impact on load reduction. Therefore, it is unlikely that best management practices effective at reducing loads during low-flow events (*e.g.,* wetlands, no-till) will be effective best management practices for reducing hormone loads. The best management practices most likely to effectively reduce hormone loads are those that can intercept exported hormones during high-flow events (*e.g.,* buffer strips) and those that reduce the input of hormones to agroecosystems (*e.g.*, reducing the hormone to macronutrient ratio of applied animal wastes).

Although lower species richness and indices of biological integrity (IBI) appeared significantly lower at in agricultural ditches impacted by drainage water from fields receiving animal wastes, variations in natural environmental parameters complicate a clear assessment of chemical exposure impacts. *In-situ* exposure studies with fathead minnows and embryos, and snapping turtles revealed no statistical differences in reproductive conditions relative to controls. Limited knowledge exists on molecular pathways associated with sex differentiation in early life stages of fathead minnows. Therefore, we developed a method to determine genetic sex in early life stage fathead minnows using a sex-linked DNA marker for which changes in the expression of genes important in sex differentiation are being exploited to more definitively evaluate effects of endocrine disrupting chemicals on early life stage fish.

Publications/Theses/Presentations:

Publications:

1. Gall, H., S. Sassman, L.S. Lee, and C. Jafvert. 2011. Hormone Chemograph Behavior in a Tile Drained Agroecosystem Receiving Animal Wastes. *Environ. Sci. Technol.*, 45:8755-8764.
2. Leet, J.K**.**, H.E. Gall, M.S. Sepúlveda. 2011. A review of studies on androgen and estrogen exposure in fish early life stages: effects on gene and hormonal control of sexual differentiation. *J. Appl. Toxicol*. 31(5): 379-398 (doi: 10.1002/jat.1682).
3. Qiao, X., N. Carmosini, F. Liu, and L.S. Lee. 2010. Probing the Primary Mechanisms Affecting the Environmental Distribution of Estrogen and Androgen Isomers. *Environ. Sci. Technol.*, 81:911-917.
4. Mashtare, M., B. Khan, and L.S. Lee. 2010. Evaluating stereoselective sorption by soils of 17α-estradiol and 17β-estradiol *Chemosphere,* 82:847–852.
5. Khan, B. and L. S. Lee. 2009. Soil Temperature and Moisture Effects on the Persistence of Synthetic Androgen 17α-Trenbolone, 17β-Trenbolone and Trendione, *Chemosphere,* 79:873-879.
6. Khan, B., X. Qiao, and L.S. Lee. 2009. Stereo-selective Sorption by Agricultural Soils and Liquid-Liquid Partitioning of Trenbolone (17α and 17β) and Trendione. *Environ. Sci. Technol.* 43:8827–8833.
7. Khan, B., S.A. Sassman, and L.S. Lee. 2008. Degradation 17α- and 17β-Trenbolone and Trendione in Agricultural Soils. *Environ. Sci. Technol.,* 42:3570-3574.

Theses:

1. Khan, Bushra**.** PhD. The environmental fate of anabolic steroid trenbolone acetate, Dissertation, Purdue University (December 2009).
2. Gall, Heather**.** PhD. Hormone Transport in a Tile-Drained Agroecosystem Receiving Animal Waste Applications, Dissertation, Purdue University (May 2011)
3. Gall*, Heather. 2008. Real-Time Monitoring and Automated Sampling of Purdue's Agricultural Fields. MS Thesis, Purdue University (December 2008).

Presentations:

1. Khan, B., Sassman, S.A. and Lee, L.S. 2007. Degradation of 17α- and 17β-Trenbolone and Trendione in Agricultural Soils, Ecological Science & Engineering Graduate Student Symposium, Purdue University, March 2007.
2. Gall, H.E., Jafvert, C.T., Lee, L.S., Jenkinson, B. "Real-Time Monitoring and Automated Sampling of Tile-Drains and Near-Field Streams," poster presented at the Ohio Valley Chapter of Science of Environmental Toxicology and Chemistry (SETAC), Oct. 3, 2008.
3. Khan, B. and L.S. Lee. 2008. Soil Temperature and Moisture Effects on Persistence of 17α-trenbolone and Trendione. SETAC North America 29th Annual Meeting, November 16-20, 2008, Tampa, FL.
4. Lee, L. S. and B. Khan. 2008. Stereo selective sorption of trenbolone and estradiol in agricultural soils (No. 61-5). 2008 Joint Meeting of The Geological Society of America, Soil Science Society of America, American Society of Agronomy, Crop Science Society of

America, Gulf Coast Association of Geological Societies with the Gulf Coast Section of SEPM, Houston, TX, USA.

5. McAlexander A, Lee L, Goforth R, Sepúlveda M. 2009. Impacts of concentrated animal feeding operations on fish communities. Joint Meeting of the Indiana Chapter of the American Fisheries Society and the Indiana Lake Management Society, January 29-31, Indianapolis, IN.
6. Leet J, Amberg J, Sepúlveda M. 2009. Sex determination in fathead minnows (*Pimephales promelas*) larvae. Department of Forestry and Natural Resources Annual Research Symposium, April 17, West Lafayette, IN.
7. Rogers S, Meyer J, Lee L, Sepúlveda M. 2009. Plasma vitellogenin levels in common snapping turtles (*Chelydra serpentina*) from concentrated animal feeding operation (CAFO) ponds versus a reference site. 2009. Department of Forestry and Natural Resources Annual Research Symposium, April 17, West Lafayette, IN.
8. Rogers S, Meyer J, Lee L, Sepúlveda M. 2009. Plasma vitellogenin levels in common snapping turtles (*Chelydra serpentina*) from concentrated animal feeding operation (CAFO) ponds versus a reference site. 2009. College of Undergraduate Research Symposium, April 21, West Lafayette, IN.
9. Rogers S, Meyer J, Lee L, Villeneuve D, Ankley, G, Sepúlveda M. 2009. Plasma vitellogenin levels in common snapping turtles (*Chelydra serpentina*) from concentrated animal feeding operation (CAFO) ponds versus a reference site. 2009. Submitted for presentation at the 30th Annual Society of Environmental Toxicology and Chemistry, November 19-23, New Orleans, LA
10. Leet J, Amberg J, Sepúlveda M. 2009. Sex determination in fathead minnows (*Pimephales promelas*) larvae. Submitted for presentation at the 30th Annual Society of Environmental Toxicology and Chemistry, November 19-23, New Orleans, LA.
11. Gall, H.E., Jafvert, C.T., Lee, L.S. Nutrient Fluxes in Agricultural Tile-Drains and Ditches. Indiana Environmental Health Summit, Indianapolis, IN.
12. Leet J, Meyer J, Goforth R, Rogers S, McAlexander A, Lee L, Villeneuve D, Ankley G, Sepúlveda M. 2010. Impacts of Land-Applied Wastes from Concentrated Animal Feeding Operations on Aquatic Organisms. FNR Annual Research Symposium, West Lafayette, IN.
13. Leet J, Lee L, Goforth R, Gott R, McAlexander A, Sepúlveda M. 2010. Impacts of Land-Applied Wastes from Concentrated Animal Feeding Operations on Aquatic Organisms. Ohio Valley Chapter of Science of Environmental Toxicology and Chemistry (SETAC), West Lafayette, IN.
14. Leet J, Amberg J, Lee L, Olmstead A, Ankley G, Sepúlveda M. 2010. Evaluation of responses to trenbolone acetate metabolites in early life-stage fathead minnows (Pimephales promelas) using molecular tools. SETAC North America 31st Annual Meeting, November 7-11, Portland, OR.
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Supplemental Keywords: hormones, manure, effluent, androgens, estrogens, CAFO, dairy, beef, poultry, swine, endocrine disruptors, aquatic species exposure, species richness, reproductive conditions

Relevant Web Sites: NA

The objective of our research is to assess the occurrence, fate and transport of natural and synthetic steroid hormones at beef and dairy cattle confined animal feeding operations (CAFOS). As part of our project, we are focusing on steroid hormone transport through surface water runoff and groundwater.

During the first year of the project, we developed and refined analytical methods for analysis of synthetic steroid hormones build and tested equipment for conducting small-scale studies and analyzed samples from several field sites.

We also assessed the potential for transport of steroid hormones in surface runoff, by analyzing runoff samples from several beef cattle operations in Northern California and conducting an experiment in which surface runoff over a manure-containing field was simulated under different conditions. Results of the field measurements and experimental study indicated that concentrations of several different steroid hormones (e.g., estrone, androstenedione and progesterone) of up to 15 ng/L can be released in runoff. This finding is potentially significant because certain steroid hormones can cause adverse impacts to fish and aquatic organisms at concentrations as low as 1 ng/L.

In the second year we focused our efforts on developing a better understanding of the processes that affect steroid fate and transport in CAFOs by conducting controlled experiments with a rainfall simulator. As part of these efforts, a small herd of cattle was maintained in a feedlot for several weeks prior to application of simulated rainfall. Runoff collected from the site was analyzed for steroid hormones, nutrients and water quality parameters. Results of the analysis indicate the presence of steroid hormone concentrations of up to 200 ng/L in the runoff. In addition, much of the mass of steroids transported from the test plots passed through a filter, indicating that it was not associated with particles that could be readily removed by settling. This observation is important because many of the best management practices for control of other contaminants (e.g., nutrients, pathogens) involve settling of particles.

During the coming year, we plan to continue our efforts to understand the transport of steroid hormones in surface runoff and groundwater. Specifically, we will conduct experiments on the release of steroid hormones in surface runoff with experiments that simulate rainfall and surface runoff on manure-containing plots. We also will collect samples of surface runoff and groundwater at field sites in California and Iowa.

Date of Report: February 16, 2012

EPA Agreement Number: 83342201

Title: Transport and Transformation of Natural and Synthetic Steroid Hormones at Beef Cattle and Dairy Confined Animal Feeding Operations

Investigators: David Sedlak[1] (PI), Thomas Harter[2] (co-PI), Edward Kolodziej[3] (co-PI)

Institutions: [1]University of California, Berkeley; [2]University of California, Davis; [3]University of Nevada, Reno

Research Category: Cooperative Agreement

Project Period: October 1, 2009-September 30, 2010

Objective of Research:

The objective of our research is to assess the occurrence, fate and transport of natural and synthetic steroid hormones at beef and dairy cattle confined animal feeding operations (CAFOs). As part of our project, we are focusing on steroid hormone transport through surface water runoff and groundwater.

Progress Summary/Accomplishments:

During the first year, we developed analytical methods, designed experiments to assess these two pathways, collected samples from several field sites and met with extension agents, ranchers and dairymen to learn more about current waste handling practices. During the second year, we finished analytical method development for synthetic steroids, developed a method for measuring particle-associated steroid hormones, built and tested rainfall simulators, and began experiments analyzing steroid transport and transformation in manure, soil, and simulated runoff from a research feedlot in Davis, CA. We also measured the steroid concentration in runoff, groundwater, and soil samples from commercial feedlots in Northern California and Iowa. During the third year, we finished the simulated runoff experiments at the research feedlot, processed several hundred soil and runoff samples to determine steroid concentration and other water quality parameters, and analyzed the data to understand how steroids were being transported and transformed on the feedlot.

Results from the simulated runoff experiments show that all of the endogenous and synthetic steroids analyzed were detected in the soil after the cattle were present at concentrations ranging from <1 ng/g to 60 ng/g. Both the endogenous and synthetic steroids in the soil were surprisingly stable over the 14 day period when the cattle were present and during the 7 days after the cattle were removed with total estrogen, testosterone, and both trenbolone isomer concentrations remaining nearly constant. Previous research has demonstrated half lives for these compounds ranging from hours to a few days, so the steroids were much more stable than expected. It is possible that the dry conditions inhibited or slowed microbial activity, or that the steroids in manure may be strongly bound to organic matter in large particles which could slow biotransformation and limit accessibility to runoff water or microbes.

Although the cattle excreted little androstenedione and progesterone, relatively high concentrations of the steroids were observed in the soil after the 14-day period when the cattle were held on the feedlot suggesting other sources or possible precursors, such as sterols in the manure and soil.

Only a small fraction of the steroids measured in the soil were released to the simulated runoff. Soil estrogen concentrations remained constant after rainfall, and TBA metabolite concentrations changed less than 10%. While the endogenous androgens and progesterone concentrations in the soil were decreased more than 80% after runoff, the mass released to runoff could only account for approximately 10% of the mass lost from the soil suggesting rapid transformation of these compounds either in the wet soil or runoff. Even the small fraction of total steroids released to the runoff caused concentrations that were, in all cases, several orders of magnitude above thresholds for feminization, masculinization, and olfactory interference. Endogenous steroid concentrations ranged between 15 and 225 ng/L with 17α-estradiol, androstenedione, and progesterone the dominant steroids present. TBA metabolite concentrations ranged between 1-390 ng/L with median concentrations of 17α-TBOH, 17β-TBOH, and TBO of 34, 16, and 19 ng/L, respectively. A significant fraction (roughly 50%) of the steroids in the runoff were associated with particles implying that treatment methods relying on particle removal could remove about half of the steroid hormones from feedlot runoff.

Results from an experiment in Iowa where feedlot runoff was treated by passing through a settling basin, vegetated infiltration basin, and a vegetated treatment area showed good removal of the endogenous steroid hormones after passing through the various treatment steps with only trace concentrations of estrone, androstenedione, and progesterone remaining in the effluent from the vegetated treatment area. These results suggest that each treatment step was effective at removing a portion of the steroid hormones, but a settling basin alone may not lower the concentrations sufficiently without further dilution.

Publications/Presentations:

Transport of Steroid Hormones by Overland Flow in Pastures and Feedlots. D. Scott Mansell, Thomas Harter, Reid Bryson, Edward P. Kolodziej and David L. Sedlak. SETAC North America 2009 Annual Meeting. New Orleans, LA November 20, 2009.

Transport of Steroid Hormones by Overland Flow from Feedlots D. Scott Mansell, Thomas Harter, Reid Bryson, Edward P. Kolodziej and David L. Sedlak. Northern California SETAC 2010 Annual Meeting. Berkeley, CA May 13, 2010.

Analytical Challenges of Trace Organics Quantification. Edward Kolodziej. Invited Presentation, NIST Emerging Contaminants Workshop, Falls Church, VA, Sept. 9, 2010.

Occurrence of Trenbolone Acetate Metabolites in Beef CAFO Soils. Stephanie Kover, Edward Kolodziej. UNR EPSCOR Summer Research Poster Presentation. August, 2010

Future Activities:

During the coming year, we plan to continue our efforts to understand the transport of steroid hormones in surface runoff and groundwater. Runoff microcosm experiments will be conducted to observe steroid stability and partitioning in runoff under various scenarios. Experiments observing TBA metabolite concentrations with time in soil samples from feedlot

pens containing TBA implanted steers will also be conducted, and leaching experiments with methanol and water will be conducted to determine what fraction of steroids will leach from soil in runoff.

Supplemental Keywords: estradiol, estrogen, endocrine disruption, trenbolone acetate, steroid, CAFO, progesterone, androstenedione, estrone, trendione, trenbolone

Relevant Web Sites: www.ce.berkeley.edu/~sedlak/; groundwater.ucdavis.edu/

Date of Report: February 21, 2012

EPA Agreement Number: 83342201

Title: Transport and Transformation of Natural and Synthetic Steroid Hormones at Beef Cattle and Dairy Confined Animal Feeding Operations

Investigators: David Sedlak[1] (PI), Thomas Harter[2] (co-PI), Edward Kolodziej[3] (co-PI)

Institutions: [1]University of California, Berkeley; [2]University of California, Davis; [3]University of Nevada, Reno

Research Category: Cooperative Agreement

Project Period: October 1, 20010-September 30, 2011

Objective of Research:

The objective of our research is to assess the occurrence, fate and transport of natural and synthetic steroid hormones at beef and dairy cattle confined animal feeding operations (CAFOs). As part of our project, we are focusing on steroid hormone transport through surface water runoff and groundwater.

Progress Summary/Accomplishments:

During the first year, we developed analytical methods, designed experiments to assess these two pathways, collected samples from several field sites and met with extension agents, ranchers and dairymen to learn more about current waste handling practices. During the second year, we finished analytical method development for synthetic steroids, developed a method for measuring particle-associated steroid hormones, and began experiments analyzing steroid transport and transformation in manure, soil, and simulated runoff from a research feedlot in Davis, CA and collected samples from commercial feedlots in Northern California and Iowa. During the third year, we analyzed the results from those experiments and began runoff microcosm experiments to assess steroid stability and transformation in feedlot runoff. During the fourth year, we conducted experiments at a commercial feedlot to determine the effect of time on TBA metabolite excretion, transformation kinetics in soil, and leaching to runoff. We also continued the runoff microcosm experiments to determine the steroid stability and partitioning in runoff.

While TBA implants are designed to provide a constant release of the steroid to the blood of the implanted animal over a 100-day period, excretion rates were not constant. Concentrations of 17αTBOH in the soil from pens containing implanted animals were, with one exception, highest (14 ng/g) in the pens where animals were implanted most recently. After 100 days, the concentrations had decreased to 2 ng/g. Other TBA metabolite concentrations in the soil also decreased throughout the 100-day lifetime of the implant. This suggests that implantation time could be a controlling factor in determining TBA metabolite concentration in soil on feedlots.

Another reason the soil concentrations of TBA metabolites decreased with time is transformation by microbes in the soil. In our experiments, we observed a half life of approximately 25 days 17αTBOH in feedlot soil. This is much slower than reported half lives in

aerobic soil of 4-50 hours, but faster than reported half lives in anaerobic soil of 260-270 days. This recalcitrance of the TBA metabolites confirms what was observed in the research feedlot experiments where the synthetic steroids were stable over the 21 day experiment even after rainfall. Time since implantation and soil transformation time could be the two most important variables in determining TBA metabolite concentrations in soil, and therefore, runoff from feedlots.

To investigate the water-extractable fraction of soil-associated steroids on CAFO surfaces, some soil samples were concurrently extracted with methanol and water. Approximately 60% of methanol-extractable steroids also were water-extractable, suggesting the existence of a strongly bound or otherwise unavailable fraction of steroids in soils. Steroids strongly associated with organic matter phases and on soil particles in locations far from water-soil interfaces are likely not available for leaching or microbial transformation.

Results from runoff microcosm experiments using endogenous steroids showed that the steroids added to the microcosms were rapidly transformed in the first several hours, but then were stable for the next 7 days. Background steroid concentrations which came from the manure were also stable over the 7 days of the experiment, although most of the concentrations are low. Androstenedione concentrations increased dramatically, increasing approximately 100%. This confirms both the production of androstenedione and the stability of the steroids observed in the previous rainfall simulator experiments on the research feedlot. The runoff, of course, has plenty of moisture which suggests the steroids are either unavailable to the microbes, or there is limited biological activity after the initial few hours. Further experiments are ongoing to determine the reason for this behavior.

Publications/Presentations:

Mansell D.S., Bryson, R.J., Harter, T., Webster, J.P., Kolodziej, Sedlak D.L., "**Fate of endogenous steroid hormones in steer feedlots under simulated rainfall-induced runoff**" *Environ. Sci. Technol*. 2011, 45:8811-8818.

Webster, J.P., Kover, S.C. Bryson, R.J., Harter, T, Mansell D.S., Sedlak D.L., Kolodziej, E.P. "**Occurrence of Trenbolone Acetate Metabolites in Simulated Confined Animal Feeding Operation (CAFO) Runoff**." *Environ. Sci. Technol*. Accepted, Under Revision, Feb. 2012

Agricultural Sources and Transformation of Steroid Hormones in Receiving Waters. Kolodziej E.P. Invited Presentation, University of Missouri. Oct. 4, 2011.

Role of Reduction-Oxidation State on the Degradation of Synthetic Growth Promoters. Kolodziej, E.P., Cwiertny, D.M., Lee, J. Invited Presentation, ACS National Meeting. Denver, CO, Aug. 28-Sept. 1, 2011.

Occurrence of Trenbolone Acetate Metabolites in Beef CAFO Soils. Stephanie Kover, Edward Kolodziej. Student World Water Forum Poster, Reno, NV, Nov. 18-19, 2010.

Occurrence of Trenbolone Acetate Metabolites in Simulated Beef Feedlot Runoff. Jackson Webster, Stephanie Kover, Edward Kolodziej. Invited Presentation, SETAC National Meeting, Portland, OR, Nov. 9, 2010.

Future Activities:

During the coming year, we plan to continue our efforts to understand the transport of steroid hormones in surface runoff and groundwater. Additional runoff microcosm experiments will be conducted to observe the effect of higher manure concentrations, equilibration time, and microbial community on endogenous steroid stability. Experiments observing the effects of moisture and shade on synthetic steroid stability in feedlot soil are also being conducted. An additional experiment observing TBA metabolite excretion in various breeds of cattle as a function of time is also be conducted. Data analysis for these experiments is ongoing.

Supplemental Keywords: estradiol, estrogen, endocrine disruption, trenbolone acetate, steroid, CAFO, progesterone, androstenedione, estrone, trendione, trenbolone

Relevant Web Sites: www.ce.berkeley.edu/~sedlak/; groundwater.ucdavis.edu/

Final Report Executive Summary

Period Covered by Report:	October 1, 2007-September 30, 2012
Date of Final Report:	December 15, 2012
EPA Agreement Number:	83342201
Title:	Transport and Transformation of Natural and Synthetic Steroid Hormones at Beef Cattle and Dairy Confined Animal Feeding Operations
Investigators:	David L. Sedlak, Thomas Harter, Edward P. Kolodziej
Institution:	UC Berkeley, UC Davis, University of Nevada
Research Category:	
Project Period:	October 1, 2007-September 30, 2012

Objective(s) of the Research Project:
The objectives of the project were to assess the occurrence, fate, and transport of synthetic and endogenous steroid hormones across major operational units (*i.e*., feedlots or corrals, storage areas/lagoons, and land application area) of beef and dairy cattle CAFOs and all major hydrologic compartments of interest: surface water runoff, soil leachate, and groundwater. The research was designed to test the overarching hypothesis that the most important hydrologic pathways for steroid releases from CAFOs are overland stormwater runoff from animal feeding areas and overflows from animal waste storage lagoons. With the exception of concentrated sources of steroids, such as animal waste lagoons, natural attenuation processes in groundwater were hypothesized to be capable of reducing concentrations of steroids below thresholds for ecological effects. The importance of surface water transport processes in determining steroid releases provides the potential for managing CAFOs in a manner that controls the release of steroids by exploiting attenuation processes that occur naturally in soil and groundwater.

To test these hypotheses, our research addressed several specific objectives. In the initial phase of the project, sensitive and accurate analytical methods were developed for quantification of synthetic steroid hormones used as growth promoters in beef cattle. Previously developed techniques for analysis of natural steroid hormones were adapted to provide accurate and precise quantification of steroids in complex matrices, such as those encountered at CAFOs. These sensitive methods were then used to assess the occurrence of synthetic and endogenous estrogens, androgens, and progestins in wastes from beef and dairy cattle CAFOs. Following characterization of the wastes, controlled runoff experiments were conducted by applying simulated rainfall to a test-plot where hormone-implanted cattle were held for several months. These data were supplemented by analysis of samples from CAFO groundwater, CAFO tile drains, and a full-scale CAFO with several runoff treatment processes as best management practices (BMPs).

Summary of Findings:
The presence of steroid hormones in runoff from CAFOs is a concern because sensitive fish species can be adversely affected by exposure to extremely low concentrations (e.g., 17β-estradiol can feminize certain fish species at concentrations below 10 ng/L). The development of a better understanding of the sources and fate of steroid hormones in CAFOs has been limited by the absence of sensitive and precise methods for quantifying steroids in a complex matrix, such as that found in manure, CAFO soils and particle-laden runoff.

One important class of steroid hormones in CAFO runoff is the synthetic androgens. Prior to our research, it was extremely difficult to detect androgens in CAFOs. To diversify the suite of analytical techniques utilized for steroid hormone analysis, a sensitive new gas chromatography/tandem mass spectrometry (GC/MS-MS) method was developed for analysis of the synthetic androgens 17α-trenbolone, 17β-trenbolone, and trendione, and melengestrol acetate in water samples. To provide a basis for monitoring steroids in manure, soil and particle-laden CAFO runoff, a solids extraction method was developed for the GC/MS-MS method for synthetic steroids and a previously developed method for natural steroids (Mansell *et al.* 2011). These accurate and precise methods provided a basis for quantitative studies of steroid source and fate at CAFOs. The methods, which are among the most sensitive and robust techniques available for analysis of these compounds, could be modified for use in routine monitoring programs or in studies designed to assess the efficacy of various best management practices.

The new analytical methods were applied to the characterization of wastes produced at CAFOs. Beef cattle treated with the synthetic androgen, trenbolone acetate, excreted the metabolites, 17α-trenbolone and 17β-trenbolone, with higher concentrations observed immediately in samples collected over a period of approximately two months after the animals were implanted. Neither trenbolone acetate nor the metabolite trendione were detected in the manure. The beef cattle also excreted natural steroid hormones, with 17α-estradiol and testosterone serving as the predominant compounds detected in fresh manure. Concentrations of naturally occurring steroid hormones detected in manure did not differ substantially from those reported for cattle that had not been implanted with trenbolone acetate (Webster *et al.* 2012.

The concentrations of steroid hormones changed after the manure was excreted. Experiments conducted at an experiment feedlot located in Davis, California, demonstrated the transformation of steroid hormones as the manure was mixed into the CAFO soil and subjected to microbial activity. Concentrations of the synthetic androgen metabolites in the CAFO soils decreased after excretion as the implants were used up and microbial transformation reactions in the soil resulted in loss of the compounds; concentrations of androgen metabolites decreased below method detection limits within 160 days after the implanted cattle were released. The natural hormones also showed evidence of transformation reactions in the CAFO soils. 17α-estradiol was converted into estrone and 17β-estradiol. Testosterone rapidly disappeared and concentrations of progesterone and androstenedione were increased on the feedlots. The rate of steroid hormone transformation was apparently affected by the moisture content of the soil; transformation rates slowed as microbial activity decreased under hot, dry summer conditions.

The analysis of manure and CAFO soils provided new insight into processes that affect steroid hormone concentrations and their potential for release to surface waters. For synthetic androgens, the metabolites, 17α-trenbolone and 17β-trenbolone are the forms of the compound most likely to be released to surface waters. If runoff from the site can be held for a period of several months after cattle receive implants, concentrations of these compounds should decrease substantially. For the natural hormones, the data indicate that estrogens are relatively stable after excretion while testosterone is rapidly transformed. Progesterone and androstenedione—two steroids that are not present at significant quantities in manure—are produced in CAFO soils as the microbes transform the steroids. The relatively high stability of these steroids suggests that they could be released to surface waters.

Following aging of the steroid hormones on the experimental feedlot, a rainfall simulator was used to mimic the conditions that occur when CAFOs are subjected to precipitation. Samples of runoff from the section of the feedlot where the rainfall simulator was being operated were collected through a movable weir structure. Samples collected at different times during the period when runoff was produced were filtered and analyzed for steroid hormones and water quality parameters. Metabolites of the synthetic androgen trenbolone acetate were detected consistently in runoff with typical concentrations ranging from 10-20 ng/L, which is comparable to concentrations of naturally occurring steroids. Attempts to close the mass balance on the androgens were complicated by difficulties in obtaining acceptable recoveries in the complex matrix. In our studies, we observed substantial differences and variability in the estimated recovery of steroids spiked in CAFO runoff for QA/QC purposes as compared to the steroids initially present in these matrices, likely due to interactions with organic matter and mineral phases or substantial biotransformation capacity. These results suggest especially challenging conditions for QA/QC assessment in agricultural runoff, and standard QA/QC procedures may not always provide accurate assessment of contaminant fate in these systems.

Nonetheless, the data suggest that the most of the metabolites remained behind in the site soils and could be mobilized in subsequent rainfall events. The natural hormones also were detected in runoff from the test plots, with concentrations typically ranging from 50-100 ng/L for 17α-estradiol, androstenedione and progesterone. Concentrations of 17β-estradiol, testosterone and estrone were considerably lower, with typical concentrations ranging from 10-20 ng/L.

One of the outcomes of these study efforts was the conceptual development of a mass balance approach that could be used to roughly predict exposure concentrations in receiving water potentially affected by animal agriculture operations. Careful comparisons of data (available in these studies and other published studies) concerning the stocking density, steroid mass excreted, soil transformation rates, and mass leached from animal wastes with projected rainfall amounts can yield estimates of steroid concentrations in runoff from animal agriculture operations. After accounting for the dilution capacity in receiving waters, it is easy to estimate the expected exposure concentration of steroids and predict the potential environmental risk arising from instances of incidental runoff. Using this approach, we propose that it is likely possible to identify receiving waters at particularly high risk for steroidal endocrine disruption (suggesting stringent runoff control and mitigation measures are necessary) or alternatively, low risk scenarios for steroidal endocrine disruption (suggesting limited runoff control or mitigation is required). This type of approach could be used to guide the implementation of runoff control and treatment technologies with respect to potential steroidal transport, as it is unlikely that a "one size fits all" approach is optimal for runoff control for animal agriculture.

Microcosm experiments consisting of manure and soil slurries amended with steroid hormones were used to provide additional insight into the microbial transformation processes occurring on feedlots. In these studies, complete transformation of testosterone and progesterone and partial transformation of 17β-estradiol within 24 hours. After 24 hours, the transformation of 17β-estradiol ceased when manure was present at concentrations typical of feedlot soils and runoff. Stabilization of 17β-estradiol may have been due to partitioning of the compound into organic matter in the manure or changes in the microbial community caused by the presence of labile organic carbon from the manure. The rate of transformation of all three classes of steroids was faster in microcosms where steroids were added to facilitate quantification, suggesting that, under field conditions, steroids may be more stable than predicted by experiments employing steroid amendments.
The microcosm studies indicated that under conditions encountered in feedlots and manure-applied fields, androgens and progesterone are likely to be transformed on the field or in runoff while estrogens likely

persist long enough to be released to surface waters.

To further assess the fate and transport of synthetic and steroid hormones, we developed a hormone transport and fate model specifically for CAFO corral surfaces, based on a previously established approach. The conceptual model considered the compacted soils underlying the unconsolidated or uncompacted soil layer that is a mix of dried manure and unconsolidated soil particles eroded from the compacted layer below and mixed into the manure through animal hoof action. The governing equations for each sub-model domain were solved numerically in the exchange layer model using the Crank-Nicolson implicit, finite-difference method. Solute concentrations for both the modeled and measured data were normalized according to the initial chemical concentration in the manure-soil layer (MSL). This conversion allowed for comparisons between the model and plot datasets since the initial concentrations, indicated by the soils analysis, were observed to vary between each plot and between solutes.

The model was calibrated against chloride data measured in the compacted soil substrate, in the manure-soil layer (MSL), and measured in surface runoff. The model reproduced the dynamics of the chloride breakthrough curve reasonably well for the runoff at the outlet of the UCD beef lot experiments. Using literature-reported sorption rates for hormones, the model was then used to predict hormone concentrations in surface runoff given the amount of precipitation during the plot experiments. While the model predicted the general dynamics of hormone transport in surface runoff, the model consistently underpredicted the high hormone concentrations observed in the field plot experiments, typically by as much as 2 orders of magnitude. The discrepancy is thought to be due to a significantly more rapid exchange of water between the USL and the free-flowing sheet flow. However, these effects also apply to salts sorbed to manure and are largely captured by the calibration process. A second, perhaps more important, factor is the transport of hormones sorbed to suspended solids. Our model did not account for such effects, which may explain the consistent underprediction by the model.

Careful comparisons of data (available in these studies and other published studies) concerning the stocking density, steroid mass excreted, soil transformation rates, and mass leached from animal wastes with projected rainfall amounts can yield estimates of steroid concentrations in runoff from animal agriculture operations. After accounting for the dilution capacity in receiving waters, it is easy to estimate the expected exposure concentration of steroids and predict the potential environmental risk arising from instances of incidental runoff. Using this approach, we propose that it may be possible to identify receiving waters at particularly high risk for steroidal endocrine disruption (suggesting stringent runoff control and mitigation measures are necessary) or alternatively, low risk scenarios for steroidal endocrine disruption (suggesting limited runoff control or mitigation is required). This type of approach could be used to guide the implementation of runoff control and treatment technologies with respect to potential steroidal transport, as it is unlikely that a “one size fits all” approach is optimal for runoff control for animal agriculture.

To verify findings from the model and test-plot study, samples were collected from full-scale feedlots and CAFOs in California and Iowa. Analysis of runoff samples collected from CAFOs in California from 2008-2011 produced findings that were consistent with data from the test-plot studies. Mean concentrations of 17α-estradiol, androstenedione and progesterone were within a factor of 3 of the mean values observed in the test-plots (i.e., 50, 310 and 120 ng/L, respectively). As expected on the basis of the test-plot results, mean concentrations of 17β-estradiol and testosterone were much lower than those of the other steroids (i.e., 12 and 3 ng/L, respectively). Concentrations of estrone were higher than expected, with a mean value (i.e., 120 ng/L) exceeding that of 17α-estradiol. A similar trend was observed in runoff from a CAFO in Iowa, with 17α-estradiol, androstenedione, progesterone dominated the profile of

steroids in the plot runoff. Partial removal of steroids was observed in a settling basin as particle-associated steroids were removed. After passage through a vegetated infiltration basin, concentrations of all of the steroids except estrone were below the method detection limit. Synthetic androgens were not analyzed at these sites because animals had not been implanted with growth hormones.

Analysis of samples from groundwater wells and a tile drain system in an area where CAFO runoff and manure was applied indicated efficient removal of steroid hormones in groundwater. Estrone and androstenedione, two of the more polar and recalcitrant steroids were detected at concentrations less than 10 ng/L were detected in fewer than 10% of the samples. Based on these results, we predict only a very limited transport potential for steroid hormones in most subsurface systems, and it is likely that subsurface transport provides an effective attenuation mechanism for most steroid hormones. Thus, it may be possible to use mitigation technologies such as riverbank infiltration, or other treatment technologies utilizing high solids concentration, biological activity, and long hydraulic retention times to attenuate steroid concentrations in high risk locations.

Publications/Presentations:

Mansell D.S., Bryson R.J., Harter T., Webster J., Kolodziej E.P. and Sedlak D.L. (2011) Fate of endogenous steroid hormones in steer feedlots under simulated rainfall-induced runoff. *Environ. Sci. Technol* 45(20): 8811-8818.

Webster J.P., Kover S.C., Bryson R.J., Harter T., Mansell D.S., Sedlak D.L. and Kolodziej E.P. (2012) Occurrence of trenbolone acetate metabolites in simulated confined animal feeding operation (CAFO). *Environ. Sci. Technol* 46(7): 3803-3810.

Parker J.A., Webster J.P., Kover S.C. and Kolodziej E.P. (2012) Analysis of trenbolone acetate metabolites and melengestrol in environmental matrices using gas chromatography-tandem mass spectrometry. *Talanta*, 99: 238-246.

Kolodziej E.P. The Occurrence and Fate of Steroidal Hormones in Surface Waters Impacted by Cattle Grazing and Animal Agriculture. USGS Seminar, Denver, CO, June 28, 2007.

Kolodziej E.P. The Occurrence and Fate of Steroidal Hormones in Surface Waters Impacted by Cattle Grazing and Animal Agriculture. American Water Resources Association Specialty Conference, Vail, CO, June 25-27, 2007.

Kolodziej E.P. Steroid Hormone Occurrence, Fate, and Transport in Northern California's Watersheds. University of California, Davis, March 17, 2008.

Kolodziej E.P. Occurrence and Fate of Steroidal Hormones in Surface Waters Impacted by Cattle Grazing and Animal Agriculture. Water and The Future of Kansas Conference, Topeka, Kansas, March 25, 2008.

Kolodziej E.P. Steroid Hormone Occurrence, Fate, and Transport in Northern California's Watersheds. University of California, Riverside, April 4, 2008.

Kolodziej, E.P., Parker, J., Webster, J.P. Solid Phase Extraction-Gas Chromatography-Tandem Mass Spectrometry Analysis of the Anabolic Steroid 17B-Trenbolone, Poster. Gordon Research Conference, Holderness, NH, June 24, 2008.

Kolodziej E.P. Solid-Phase Extraction-GC/MS-MS Analysis of the Anabolic Steroid 17β-Trenbolone. SETAC National Meeting, Tampa Bay, FL, November 17, 2008.

Kolodziej E.P., Harter, T., Sedlak, D.L. Transport and Transformation of Steroid Hormones at Beef and Dairy CAFOs" EPA STAR Progress Meeting, Tampa, FL, November 16, 2008.

Mansell D.S. and Sedlak D.L. Transport of steroid hormones by overland flow from pastures and feedlots. NorCal SETAC Annual Meeting, Davis, CA, April 21, 2009.

Kolodziej E.P. Gas Chromatography-Tandem Mass Spectrometry Analysis of Growth Promoting Synthetic Steroid Hormones in Environmental Matrices. SETAC North America 2009 Annual

Meeting. New Orleans, LA. November 20, 2009.
Mansell D.S., Harter T., Bryson R., Kolodziej, E.P. and Sedlak D.L. Transport of Steroid Hormones by Overland Flow in Pastures and Feedlots. SETAC North America 2009 Annual Meeting. New Orleans, LA November 20, 2009.
Mansell D.S. and Sedlak D.L. Transport of steroid hormones on concentrated animal feeding operations. SETAC Annual Meeting, Berkeley, CA, May 3-4, 2010.

Supplemental Keywords: estradiol, estrogen, endocrine disruption

Relevant Web Sites: www.ce.berkeley.edu/~sedlak/; groundwater.ucdavis.edu/; http://www.unr.edu/cee/faculty/ed-kolodziej.html

This photograph illustrates one of the field sites in Northern California where runoff was collected.

This photo shows the test-plot being subjected to simulated rainfall during the runoff studies.

This photograph illustrates the surficial soil and underlying clay layer at the test-plot where controlled runoff studies were conducted. Water and steroid hormones did not leach to groundwater due to the low-permeability clay layer.

Part 1: Web Summary Report

NCER Assistance Agreement Annual Report Summary

Period Covered by Report: 1/01/2010-6/30/2011

Date of Report: 11/10/2011

EPA Agreement Number: R833423

Title: Effects of Cattle Manure Handling and Management Strategies on Fate and Transport of Hormones in the Feedlot and the Field

Investigators: Daniel D. Snow, Shannon Bartelt-Hunt, Bill Kranz, Terry Mader, Charles Shapiro, David Shelton

Institution: University of Nebraska-Lincoln

Project Period: 07/01/2007-06/30/2011

Project Amount: $699,997

RFA: Fate and Effects of Hormones in Waste from CAFOs, Endocrine Disruptors, EPA-G2006-STAR-M1

Research Category: Endocrine Disruptors

Objective of Research: The objectives of this research project are to (1) quantify hormones in various stages of the manure pathway in cattle feedlots, (2) determine the effects of different handling practices of cattle feedlot wastes on the stability and availability of hormones in cattle feedlots, (3) determine the effects of different land application strategies on the fate and transport of hormones used in beef cattle production in vadose zone soils, and (4) determine if grasses from conservation buffers assimilate hormones.

Progress Summary/Accomplishments: Results of the feeding pen run-off studies were presented in late 2009 (Snow et al 2009) and the data used to prepare a manuscript for submission to *Environmental Science and Technology* (Bartelt-Hunt et al 2011). Briefly, the 2-year controlled study evaluated the occurrence of sixteen natural and synthetic steroid hormones and metabolites in rainfall runoff as well as in manure and soil collected from feedlot surfaces from beef cattle feedlots from both treated and untreated animals. Samples were extracted and analyzed for metabolites of the synthetic androgen trenbolone acetate, 17α-trenbolone, 17β-trenbolone, for the non-steroidal semi-synthetic estrogen agonist, α-zearalanol, and the synthetic progesterone melengesterol acetate, as well as a wide range of naturally-occurring estrogens, androgens, and *fusarium* metabolites. Traces of synthetic steroids including trenbolone metabolites and melengestrol acetate were detected in fresh manure and in feedlot surface soils from pens holding cattle administered implants. Traces of melengesterol acetate were detected in 6% of runoff samples from feedlots holding cattle administered synthetic steroids. Synthetic steroids were not detected in manure or runoff from control cattle. A wide range of natural hormones and related compounds were detected in runoff and feedlot surface soils and manure

from cattle given synthetic steroids and from control cattle, with no statistically significant differences in concentration. These results indicate that runoff from confined animal production facilities is of environmental and public health concern regardless of the use of growth promotants. The analytical methods developed for all tasks conducted as part of this project were described and submitted for publication to *Analytical and Bioanalytical Chemistry* (Snow et al 2011).

Results from the 2008 crop simulated rainfall run-off experiments were summarized in a conference proceedings (Biswas et al 2011). Additional rainfall simulation experiments were conducted in early summer 2009 using composted and stockpiled manure from the 2008 feeding studies. These results are currently being evaluated and compared to the previous year. Grassed buffers fertilized with stockpiled manure from 2007 were sampled intermittently during 2009-10 for evaluation of uptake potential in plants. Soil pore water was also sampled using lysimeters beneath crops fertilized with manure for determination of steroid hormone leaching potential. Split samples from the lysimeter site have been collected and sent to US EPA Kerr Research Laboratory for nutrients, major ions, and estrogen conjugates. The remaining survey task samples were collected in late 2010 and in 2011 with the assistance of scientists from the Kerr Research Laboratory.

Laboratory tests were completed to measure degradation rates, sorption and transformation products using ^{14}C labeled testosterone and 17beta-estradiol. The results from the bioreactor and sorption-desorption experiments provided the basis for 2 master's-level student research projects and will be reported in journal articles. A PhD student (Sagor Biswas) in the UNL Biosystems Engineering Department will use the data from the rainfall simulation experiments, lysimeter leaching and survey tasks as part of his PhD dissertation. He is also planning to use APECS flow models with data from the rainfall simulation studies to make predictions about the fate and transport of steroid hormones from cattle manure in agricultural watersheds. Results of all of these studies will be reported in journal articles or conference proceedings.

Publications

Bartelt-Hunt, S.L., D.D. Snow, W.L. Kranz, T.L. Mader, C.A. Shapiro, S.J. van Donk, D.P. Shelton, D.D. Tarkalson, and T.C. Zhang. 2011. Effect of growth promotants on the occurrence of steroid hormones on feedlot soils and in runoff from beef cattle feeding operations. *Environmental Science and Technology,* accepted.

Ma, R., T.C. Zhang, S.L. Bartelt-Hunt, B. Kranz, D.D. Snow, T. Mader, C. Shapiro, D.P. Shelton, S. J. van Donk, D.D. Tarkalson, and S. Ensley. 2011. Fate and Transport of Testosterone in Subsurface Soils: Sorption and Desorption Tests, *Environmental Science and Technology,* In preparation.

Snow, D.D., T. Damon-Powell, S. Onanong, and D.A. Cassada. 2011. Sensitive and simplified analysis of natural and synthetic steroids in water and solids using on-line solid phase extraction

and microwave-assisted solvent extraction coupled to liquid chromatography tandem mass spectrometry atmospheric pressure photoionization. *Analytical and Bioanalytical Chemistry* (In review).

Presentations/conference proceedings

Bartelt-Hunt, S.L. D.D. Snow, S. DeVivo, T.C. Zhang, B. Kranz, T. Mader, C. Shapiro, D. Shelton, S. Van Donk, S. Ensley. and D. Tarkalson. 2009. Influence of Composting on Hormone Persistence in Beef Cattle Manure, Abstracts with Programs, Hormones in the Environment Session. Society of Environmental Toxicologists and Analytical Chemists. SETAC North America 30th Annual Meeting, New Orleans, LA

Snow, D.D., S.L. Bartelt-Hunt, T.C. Zhang, B. Kranz, T. Mader, C. Shapiro, D. Shelton, S. Van Donk, S. Ensley. and D. Tarkalson. 2009. Dissolved Steroid Hormones in Surface Run-off from Cattle Feeding Pens. In Abstracts with Programs, Hormones in the Environment Session. Society of Environmental Toxicologists and Analytical Chemists. SETAC North America 30th Annual Meeting, New Orleans, LA.

Biswas, S. W.L. Kranz, S.L. Bartelt-Hunt, T.L. Mader, C.A. Shapiro, D. P. Shelton, D. D. Snow, D.D. Tarkalson, S.J. van Donk, T.C. Zhang, S.Ensley. 2011. Feedlot Manure Handling and Application Strategies on Surface Runoff of Artificial Hormones Applied to Rowcrop Fields. ASABE Annual International Meeting, Gault House, Louisville, Kentucky, August 7 – 10, 2011.

Master's Theses

Devivo, S. 2009. The effects of waste handling practices on the fate of androgens and estrogens from concentrated animal feeding operations. M.S. Thesis. University of Nebraska—Lincoln. 107 p.

Ma, Rui. 2009. Sorption and desorption of testosterone in agricultural soils. M.S. Thesis. University of Nebraska--Lincoln, 97 p.

Awards – University of Nebraska College of Engineering Multidisciplinary Research Award- EPA CAFO Project (2010)

Future Activities: The remaining samples from rainfall simulation experiments, lysimeter plots, and survey tasks have been analyzed. Additional samples for the survey task are being analyzed to characterize hormone levels in soils receiving manure applications and feeding pens at other research farms. Additional manuscripts are planned to summarize the data from both the 2008 and 2009 rainfall simulation experiments, composting results, lysimeter leaching, and grass uptake studies with all sampling and analysis to be completed in mid-2011.

Supplemental Keywords: estradiol, estrogen, androgen, endocrine disruption; Scientific Discipline, Health, RFA, Endocrine Disruptors - Environmental Exposure & Risk,

Environmental Chemistry, Endocrine Disruptors - Human Health, animal feeding operations, endocrine disrupting chemicals, EDCs, CAFOs, agrochemicals

Relevant Web Sites:

http://snr.unl.edu/people/faculty/snow-dan.asp

http://watercenter.unl.edu/

NCER Assistance Agreement Annual Report Summary

Date of Report: 12/18/2009

EPA Agreement Number: R833423

Title: Effects of Cattle Manure Handling and Management Strategies on Fate and Transport of Hormones in the Feedlot and the Field

Investigators: Daniel D. Snow, Shannon Bartelt-Hunt, Bill Kranz, Terry Mader, Charles Shapiro, David Shelton

Institution: University of Nebraska-Lincoln

Research Category: Fate and Effects of Hormones in Waste from CAFOs, Endocrine Disruptors, EPA-G2006-STAR-M1

Project Period: 07/01/2008-06/30/2009

Objective of Research: The objectives of this research project are to (1) quantify hormones in various stages of the manure pathway in cattle feedlots, (2) determine the effects of different handling practices of cattle feedlot wastes on the stability and availability of hormones in cattle feedlots, (3) determine the effects of different land application strategies on the fate and transport of hormones used in beef cattle production in vadose zone soils, and (4) determine if grasses from conservation buffers assimilate hormones.

Progress Summary/Accomplishments: Approximately 900 samples of surface run-off, feedlot surfaces and soil samples have been collected for this project since 2007. Two cattle feeding studies were completed in 2007 and 2008 to provide manure from treated and untreated cattle. Samples of feeding pen surfaces and run-off samples collected for analysis of steroid hormones. Feedlot surface samples collected at 7, 45 and 125 days after cattle placed in pens. Five run-off events were sampled in 2007 and nine events in 2008. Manure from the first study was placed in compost piles and anaerobic stockpiles, covered and sampled over 6-8 month period and sampled regularly to determine persistence during manure storage under differing conditions.

Stockpiled and composted manure was applied during the summer to tillage plots watered with simulated rain-fall for crop run-off tests. Additional rainfall simulation experiments were conducted in early summer 2009 using composted and stockpiled manure from the 2008 feeding studies. Grassed buffers fertilized with stockpiled manure from 2007 have been sampled for evaluation of uptake potential in plants. Soil pore water was sampled using lysimeters beneath crops fertilized with manure for determination of steroid hormone leaching

potential. Split samples from the lysimeter site have been collected and sent to US EPA Kerr Research Laboratory for nutrients, major ions, and estrogen conjugates.

Laboratory tests are underway to determine degradation rates, sorption and transformation products using ^{14}C labeled testosterone and 17beta-estradiol. Batch sorption-equilibration experiments were conducted with soils collected from 2 of the 4 research sites to determine conditions under which these compounds may be expected to be mobilized. The results from the bioreactor and sorption-desorption experiments provided the basis for 2 master's-level student research projects in Civil Engineering.

Publications/Presentations:

Ma, Rui, T. C. Zhang, L. Bartelt-Hunt, D. Snow, B. Kranz T. Mader, C. Shapiro, D. Shelton, S. Van Donk, S. Ensley, and D. Tarkalson.Field-and Laboratory-Scale Studies on Fate and Transport of Hormones in Soil/Water System. Abstracts. American Society of Civil Engineers, Annual Meeting, Kansas City, MO. Kansas City, MO, March 31, 2009; American Society Civil Engineers, Kansas City, Missouri, 2009.

Ma, Rui, Bartelt-Hunt, S.L.; T.C. Zhang, D. Snow, B. Kranz, T. Mader, C. Shapiro, D. Shelton, S. Van Donk, S. Ensley, D. 2009. Sorption and Desorption of Testosterone in Agricultural Soils, Abstracts. American Society of Civil Engineers, Annual Meeting, Kansas City, MO.

Bartelt-Hunt, S.L.; S. L. DeVivo, T.C. Zhang, D. Snow, B. Kranz, T. Mader, C. Shapiro, D. Shelton, S. Van Donk, S. Ensley, D. Tarkalson. 2009. Influence of Composting on Hormone Persistence in Beef Cattle Manure, Abstracts, Association of Environmental Engineering and Science Professors, Annual Meeting, Iowa City, IA.

Bartelt-Hunt, S.L. D.D. Snow, S. DeVivo, T.C. Zhang, B. Kranz, T. Mader, C. Shapiro, D. Shelton, S. Van Donk, S. Ensley. and D. Tarkalson. 2009. Influence of Composting on Hormone Persistence in Beef Cattle Manure, Abstracts with Programs, Hormones in the Environment Session. Society of Environmental Toxicologists and Analytical Chemists. SETAC North America 30th Annual Meeting, New Orleans, LA

Sellin, M.R., D.D. Snow, and A.S. Kolok. Endocrine disruption in an agricultural watershed: The role of sediments, 2009. In: Hormones in the Environment Session. Abstracts with Programs, Society of Environmental Toxicologists and Analytical Chemists. SETAC North America 30th Annual Meeting, New Orleans, LA.

Snow, D.D., S.L. Bartelt-Hunt, T.C. Zhang, B. Kranz, T. Mader, C. Shapiro, D. Shelton, S. Van Donk, S. Ensley. and D. Tarkalson. 2009. Dissolved Steroid Hormones in Surface Run-off from Cattle Feeding Pens. In Abstracts with Programs, Hormones in the Environment Session. Society

of Environmental Toxicologists and Analytical Chemists. SETAC North America 30th Annual Meeting, New Orleans, LA.

Future Activities: The remaining samples from the rainfall simulation experiments, lysimeter plots, and survey task will be analyzed. Additional samples for the survey task will be collected to characterize hormone levels in soils receiving manure applications and feeding pens at other research farms. Papers describing the results for the batch equilibration studies, composting experiments, and feeding pen run-off studies are currently being drafted. Data on initial rainfall simulation studies and lysimeter plots will be presented at conference next year. A third graduate student in Biosystems Engineering is working on developing preferential flow models using the data generated from this study to make predictions about the fate and transport of steroid hormones from cattle manure in agricultural watersheds.

Supplemental Keywords: estradiol, estrogen, androgen, endocrine disruption; , Scientific Discipline, Health, RFA, Endocrine Disruptors - Environmental Exposure & Risk, Environmental Chemistry, Endocrine Disruptors - Human Health, animal feeding operations, endocrine disrupting chemicals, EDCs, CAFOs, agrochemicals

Relevant Web Sites:

http://snr.unl.edu/people/faculty/snow-dan.asp

http://watercenter.unl.edu/

NCER Assistance Agreement Annual Report Summary

Date of Report: 5/20/2009

EPA Agreement Number: R833423

Title: Effects of Cattle Manure Handling and Management Strategies on Fate and Transport of Hormones in the Feedlot and the Field

Investigators: Daniel D. Snow, Shannon Bartelt-Hunt, Bill Kranz, Terry Mader, Charles Shapiro, David Shelton

Institution: University of Nebraska-Lincoln

Research Category: Fate and Effects of Hormones in Waste from CAFOs, Endocrine Disruptors, EPA-G2006-STAR-M1

Project Period: 07/01/2007-06/30/2008

Objective of Research: The objectives of this research project are to (1) quantify hormones in various stages of the manure pathway in cattle feedlots, (2) determine the effects of different handling practices of cattle feedlot wastes on the stability and availability of hormones in cattle feedlots, (3) determine the effects of different land application strategies on the fate and transport of hormones used in beef cattle production in vadose zone soils, and (4) determine if grasses from conservation buffers assimilate hormones.

Progress Summary/Accomplishments: Analytical methods for extraction and analysis of trace levels of steroid hormones from water and soils were developed. Detection limits were validated using replicate analysis of low-level fortified matrix. Two cattle feeding studies were completed in 2007 and 2008 to provide manure from treated and untreated cattle. Samples of feeding pen surfaces and run-off samples collected for analysis of steroid hormones. Feedlot surface samples collected at 7, 45 and 125 days after cattle placed in pens. Approximately 30 runoff events sampled using tipping bucket samplers from the feeding pens. Cattle treated with implants gained 3.23 lb/day while untreated cattle gained 2.65 lb/day. Manure from the first study was placed in compost piles and anaerobic stockpiles, covered and sampled over 6-8 month period to determine persistence during manure storage.

Stockpiled and composted manure was applied during the summer to tillage plots watered with simulated rain-fall for crop run-off tests. Grassed buffers fertilized with stockpiled manure have been sampled for evaluation of uptake potential in plants. Soil pore water is currently being sampled beneath crops fertilized with manure for determination of steroid hormone leaching potential. Laboratory tests are underway to determine degradation rates, sorption and

transformation products using ^{14}C labeled testosterone and estradiol. Preliminary results suggest that supplement concentrations are much lower than the levels of natural (endogenous) steroid hormones in run-off water.

A water resources and irrigation engineer, located at the University of Nebraska West Central Research and Extension Center, was added as a co-principal investigator on the project to provide oversight for the lysimeter studies conducted in North Platte, Nebraska. A revised quality assurance project plan was prepared and submitted to the program officer in April 2008 following development of standard operating procedures under the project and detailed descriptions of the survey task, sprinkler irrigation and batch sorption testing.

Publications/Presentations:

Snow, D., S. Bartelt-Hunt, B. Kranz, T. Mader, C. Shapiro, D. Shelton, and T. Zhang. 2007. Effects of Cattle Manure Handling and Management Strategies on Fate and Transport of Hormones in the Feedlot and the Field (EPA RD833423). Proceedings of the Fate and Effects of Hormones in Waste From Concentrated Animal Feeding Operations (CAFOs) Workshop. August 20-22, 2007, EPA Region V Conference Facility. Chicago, IL.

Snow, D.D., S. Bartelt-Hunt, B. Kranz, T. Mader, C. Shapiro, D. Shelton, T. Zhang, S. van Donk, D. Tarkalson, S. Ensley, (2008) EPA CAFO Project "Effects of Cattle Manure Handling and Management on Fate and Transport of Hormones in the Feedlot and the Field" Heartland Regional Water Coordination Initiative. Heartland Animal Manure Management Workshop, October 1-2, 2008 Stoney Creek Inn, St. Joseph, MO.

Snow, D., S. Bartelt-Hunt, B. Kranz, T. Mader, C. Shapiro, D. Shelton, S. van Donk, and T. Zhang. 2007. Effects of Cattle Manure Handling and Management Strategies on Fate and Transport of Hormones in the Feedlot and the Field – Project Update (EPA RD833423). Endocrine Disruptors In the Environment – A U.S. EPA Science To Achieve Results (STAR) Progress Review November 16, 2008, Tampa Marriot Waterside Hotel, Tampa, FL.

Future Activities: Tasks and activities underway during the second year of the project include rainfall simulation experiments with first year stockpiled and composted manure, sampling of lysimeters beneath test plots receiving manure from the first year pen study, sampling of grasses fertilized with manure, laboratory degradation studies using bioreactors, sorption and degradation experiments with ^{14}C-labelled compounds, and analysis of first year feedlot run-off, pen surface and rain-fall simulation samples. The survey task will be scheduled during the third year of the project in order to spread out sample collection and analysis, as well as to help coordinate cooperative efforts with the EPA ORD National Risk Management Research Laboratory to include samples of ground water from the project sites.

Supplemental Keywords: estradiol, estrogen, androgen, endocrine disruption; , Scientific Discipline, Health, RFA, Endocrine Disruptors - Environmental Exposure & Risk, Environmental Chemistry, Endocrine Disruptors - Human Health, animal feeding operations, endocrine disrupting chemicals, EDCs, CAFOs, agrochemicals

Relevant Web Sites:

http://snr.unl.edu/people/faculty/snow-dan.asp

http://watercenter.unl.edu/

Final Report Executive Summary

Period Covered by the Report: 7/1/2007-6/30/2011

Date of Final Report: 12/20/2011

EPA Agreement Number: R833423

Title: Effects of Cattle Manure Handling and Management Strategies on Fate and Transport of Hormones in the Feedlot and the Field

Investigators: Daniel D. Snow, Shannon Bartelt-Hunt, Bill Kranz, Terry Mader, Charles Shapiro, David Shelton

Institution: University of Nebraska-Lincoln

Research Category: Fate and Effects of Hormones in Waste from CAFOs, Endocrine Disruptors, EPA-G2006-STAR-M1

Project Period: 7/1/2007-6/30/2011

Objective(s) of the Research Project: The objectives of this research project are to (1) quantify hormones in various stages of the manure pathway in cattle feedlots, (2) determine the effects of different handling practices of cattle feedlot wastes on the stability and availability of hormones in cattle feedlots, (3) determine the effects of different land application strategies on the fate and transport of hormones used in beef cattle production in vadose zone soils, and (4) determine if grasses from conservation buffers assimilate hormones.

Summary of Findings: This project was organized into several tasks directed at the objectives above and to provide some direct assessment of the occurrence fate and transport of natural and synthetic steroid hormones and growth promoters in cattle waste. Effects of manure management (stockpiling versus composting) and tillage practices on steroid hormone fate and occurrence were evaluated through both field and laboratory experiments. Hundreds of water, soil and plant samples were collected and analyzed to evaluate possible impacts from natural and synthetic steroid hormones during manure collection and application in agricultural settings. A major task involved increasing our understanding of hormone occurrence and potential losses to run-off in cattle feeding operations. Two separate controlled studies show that comparable levels of endogenous hormones, steroid transformation products, and a variety of related compounds are likely to likely to occur in manure and feedlot run-off. Growth promoting compounds also occur on feedlot surfaces and may be transport through run-off, though the levels decrease over time after treatment especially in surface environments. The effect of manure stockpiling versus composting on steroid hormone levels was evaluated using manure from each feeding pen study. In general, concentrations of extractable steroid hormones decreased during both stockpiling and composting though the latter seems to provide a better environment for transformation. Experiments with laboratory bioreactors confirm that specific steroid hormones are degraded

more rapidly under aerobic conditions. The results suggest that manure composting, often used as a means to improve the quality of the material for use as a soil conditioner, also can be explored as a means to reduce release of hormones to the environment. Because hormones can attach to soil particles the effect of sorption and desorption was evaluated through additional laboratory experiments. Degradation of steroid hormones in soils and manure will be affected by degree and rate of adsorption and desorption. The sorption experiments indicate that desorption is important in determining the mobility and presumably degradability of these compounds in the environment.

A second major effort was directed at examining the losses of steroid hormones from soils fertilized with manure under differing tillage practices. The manure used in each set of crop surface run-off experiments had been composted or stockpiled for several months prior to its use in the tillage effect experiments. Steroid hormone levels in this material, applied at agronomic rates consistent with best management practices, were greatly reduced over freshly deposited manure. Only a small fraction of the samples collected and analyzed in the first year's crop run-off studies contained detectable levels of any of the compound measured. Because of this, the relationship of tillage practices (incorporation versus surface application) could not be accessed from these experiments. The following year, smaller scale run-off experiments were conducted with the additional application of a synthetic estrogen to help evaluate potential losses of steroid hormones. Though not manure-borne, the synthetic compound would be expected to behave in a similar manner to natural estrogens. Concentrations of the synthetic estrogen were easily measured in the second set of run-off experiments and showed significant differences between incorporated and surface applied manure. Traces of several other steroid hormones were detected and were generally higher in the runoff from the surface applied test plots. Though site specific, these results suggest incorporation may be explored as a method for reducing losses of manure-associated steroid hormones in surface run-off from fields receiving manure. Because the concentrations measured were exceedingly low regardless of the treatment, however, additional work is needed to establish this as a recommended management practice.

Vertical leaching of hormones over time from soils was examined using additional test plots fertilized with stockpiled or composted manure and planted with wheat. These plots received portions of the same stockpiled or composted manure containing low steroid hormone levels used in the first run-off simulation study. An inert tracer, added to measure vertical transport times from the surface, was observed in pore water samples collected from beneath the test plots approximately 1-year after manure application. Most of the samples collected over the study period did not have measurable levels of steroid hormone, though a few compounds were briefly detected at approximately the same time as the tracer. Because the levels in the applied manure were already quite low, it is likely that dilution and/or degradation at the surface resulted in undetectable levels of hormones below the crop rooting zone. In contrast to hormones, levels of nitrate and other nutrients increased in the pore water at approximately the same time as the inert tracer indicating other contaminants may be transported from the surface. The final objective of this research was to evaluate plant uptake of manure-borne steroid hormones in grasses fertilized with manure. The task associated with the objective used stockpiled manure collected from the first feeding pen study to fertilize warm and cool season grasses commonly used in crop buffers. As with the leaching study, this manure was applied at agronomic rates and had relatively low

levels of steroid hormones in comparison to freshly deposited cattle waste. Few papers have described analysis of grasses for steroid hormones and the laboratory methods proved to be more challenging than even the complex manure matrix. Though significant levels of some steroid hormones were detected in the grasses, it s difficult to determine if these are derived from manure application or from other sources. Recent research suggests that mammalian hormones, along with phytoestrogenic compounds, may occur naturally in plants. No samples provided evidence of uptake of synthetic steroid hormones by the grasses.

Publications/Presentations:

Journal Articles

In preparation

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Relevant Web Sites: